AMENDMENT No. 1 TO SPECIFICATION

METHOD OF INHIBITING OPIOID TOLERANCE WITH CHIMERIC HYBRID ANALGESICS

Cross-Reference to Related Applications

This is a division of U.S. Patent No. 6,881,829 ~~application Serial No. 10/134,187~~, filed 04/26/02, as to which Applicant elected a restriction of the invention as required by an Office Action mailed on 09/23/03.

Statement Regarding Federally Sponsored Research or Development

Not applicable.

Reference to Sequence Listing, a Table, or a Computer Program Listing Compact Disk Appendix

A written Sequence Listing and a computer readable form of the sequence listing, consisting of one file named ChimericHybridAnalgesics.ST25.txt on one disk, are attached as Appendices.

Background of the Invention

Field of the Invention. The present invention lies firmly within the fields of drug, bio-effective and body treating compositions, more specifically methods for acute and chronic pain relief.

Description of the Prior Art. The present invention relates to inhibiting the development of opioid tolerance in the treatment of pain by using novel hybrid alkaloid/peptide chimeric molecules. The relief of suffering due to pain is an important objective of clinical practice and for restoring quality to life and the ability to function normally to pain sufferers.

Pain represents an integrated, complex, perception of noxious stimuli originating from somatic elements such as arms and legs and/or from visceral organs such as heart and liver. Mechanistically, acute pain signaling involves noxious stimulation of free nerve endings innervating somatic elements and/or visceral organs leading to the activation of different types of slowly-conducting afferent fibers of the A delta and C classes, terminating in the dorsal sensory spinal cord. A significantly more complex etiology underlies the initiation and persistence of chronic pain syndromes. This involves initial damage to peripheral nerves innervating somatic and visceral fields, persistent immunological challenge by cytokines and inflammatory mediators, reorganization of spinal cord and brainstem relay systems, and higher cortical adaptation.

From an established pharmacological perspective, opioids remain the key agents of choice for treatment of a wide variety of acute and chronic pain states. The prototype opioid analgesic or painkiller is morphine. Morphine and morphine-related opioids produce their painkilling effects by profound pharmacological inhibition of neurons of the peripheral/sensory nervous system (PNS) and the central nervous system (CNS). The biochemical and cellular effects of morphine, including profound analgesia, are transduced through a membrane-associated G-protein designated the mu (μ) opioid receptor (MOR), found in high concentrations within the PNS and CNS.

Unfortunately, the high degree of pain relief afforded by morphine and similar opioid compounds is associated with many undesirable side effects, all mediated through activation of the MOR. They include drowsiness, nausea, emesis (vomiting), changes in mood (dysphoria), respiratory depression, decreased gastrointestinal motility (constipation), pruritis (itching), alterations in endocrine and autonomic function, and physical and psychological dependence leading to addiction.

In addition to the adverse physiological sequelae listed above, a major associated risk is that repeated daily administrations of morphine or morphine-like opioids will eventually induce

significant tolerance to the therapeutic effects of the drug as well as initiating some degree of physical dependence. Here opioid tolerance is operationally defined as an escalating dosage regimen required to achieve the same magnitude of pain relief over a defined time course.

The administration of escalating dosage so as to achieve the same magnitude of pain relief can increase the likelihood and the severity of undesirable side effects such as drowsiness, nausea, emesis (vomiting), changes in mood (dysphoria), respiratory depression, decreased gastrointestinal motility (constipation), pruritis (itching), alterations in endocrine and autonomic function.

The degree of tolerance and physical dependence will vary with the particular opioid employed, the correlation with MOR-selective opioids such as morphine being high, the frequency of administration, and the quantity of opioid administered.

In a wide variety of clinical indications requiring prolonged use of opioids, tolerance induction and addiction are closely linked, with the development of physical and psychological dependence always a major concern. Addiction with physical dependence can be

difficult to treat due to the effects of withdrawal associated with dependence.

From an established clinical perspective, when morphine and/or similar opioid analgesics are administered, the treating health care provider must recognize that only symptomatic treatment of pain is being provided. The health care provider must therefore constantly weigh the benefits of this immediate (day by day) relief against its costs and risks to the patient. Accordingly, a decision to relieve the chronic pain in particular clinical situations via administration of current opioid analgesics may be short sighted and an actual disservice to the patient.

Morphine and related MOR-selective opioids also relieve suffering by ameliorating the emotional or affective component of the painful experience. Consequently, if little or no external emotional support is provided, for example by biofeedback procedures or cognitive behavioral therapy, some patients may require considerably more than the average dose of an opioid to experience any relief from pain; similarly, others may require more frequent administration. These are major factors supporting the use of patient-controlled analgesia (PCA) for acute post-operative pain control, where the affective aspects of painful experience are successfully addressed. In effect, many health

care providers frequently tend to prescribe therapeutic dosages of opioids that are either too low and/or administered at infrequent time intervals out of an exaggerated concern for minimizing addiction potential. The resultant therapeutic regimen fails to provide adequate analgesia over time.

In light of the caveats listed above, many health care providers are constantly encouraged to employ measures other than opioid drugs to relieve chronic or acute pain, even when such alternative methods show limited efficacy in the absence of opioid therapy. These typically include the use of local nerve block, combinations of antidepressant and anticonvulsant CNS drugs, electrical stimulation, acupuncture, hypnosis, or behavioral modification (Reuler et al., Ann. Intern. Med. 93:588-596 (1980)). Additionally, many practitioners respond to their patients' continued complaints of inadequate pain relief with even more exaggerated concerns about dependency. This is done despite the high probability that the request for more opioid is only the expected consequence of the inadequate dosage originally prescribed. (Sriwatanakul et al., J.A.M.A. 250:926-929 (1983))

It has also been documented that children are probably more apt to receive inadequate dosages for pain than are adults based on the

same type of reasoning concerning tolerance and dependence Schechter. (N. L., Curr. Probl. Pediatr. 15 (1985))

Finally, it is useful to remember that the typical initial dose of morphine (10 mg/70 kg body weight) relieves post-operative pain satisfactorily in only about two-thirds of patients. (See page 511, Goodman & Gilman, The Pharmacological Basis of Therapeutics, 7th Ed.)

Morphine/opioid-induced physiological and psychological side effects pose major obstacles to their unfettered, widespread usage as the mainspring therapeutic regimen for pain relief across clinical populations in the United State and abroad. Intrinsic issues of opioid safety and efficacy were addressed by a prior invention (U.S. Patent 5,891,842), where I established a therapeutic procedure or treatment regimen for inducing or eliciting a markedly enhanced opioid-dependent analgesic response within a living subject. That treatment methodology employs the concurrent administration of two recognized, self-contradicting and physiologically antagonistic compounds, the opioid analgesic morphine sulfate and the tachykinin peptide substance P (SP), at individual concentrations that had been empirically shown to have either marginal or completely ineffectual pharmacological properties *in vivo*. Because noxiously challenged or damaged

sensory nerves release a variety of excitatory chemical mediators, including SP, the tachykinin SP had been previously designated as a nociceptive or pain-producing peptide transmitter at the spinal level. Nevertheless, my research demonstrates that at prescribed low nanogram concentrations SP appears to be a potent regulator of opioid analgesia *in vivo*. Despite this apparent contradiction and the previously demonstrated physiological antagonism between these compounds in their traditional formats and conventionally used concentrations, my novel treatment process demonstrated a synergistic relationship over a period of time, and that an effective and efficacious opioid-induced analgesia results within the living subject from the process.

Unfortunately, because my prior invention requires the concurrent administration of two different self-contradicting and physiologically antagonistic compounds, SP and morphine, it presents difficulties in successfully establishing and testing the appropriate concurrent dosages for efficacious and safe administration in humans, as reflected by FDA and NIH clinical testing guidelines.

While morphine is the prototype opioid analgesic or painkiller, its complex alkaloid characteristics differ greatly from those of peptides, and SP is a peptide. In subsequent research, therefore,

collaborators and I combined the active pharmacological domains of SP and the peptide endomorphin-2 into one chemical entity: a novel seven amino acid peptide chimera, designated ESP7. Repeated administration of the chimeric molecule into the rat spinal cord milieu produced analgesia mediated by the MOR without a loss of potency over a 5-day time course. Essentially, ESP7 represented a non-tolerance forming compound with future potential as a specialized spinal analgesic for control of acute and/or chronic pain. (Foran, et al., A Substance P-opioid chimeric peptide as a unique non-tolerance-forming analgesic, 97 Proceedings of the National Academy of Sciences 13 (2000))

Although ESP7 provided the advantage of a single analgesic molecule, it has several unfortunate disadvantages. Operationally, the peptide chemical nature of ESP7 restricts its effective dosage and time-effect relationship within the CNS due to significant metabolism in the blood stream. This is supported by collected pharmacological data indicating significant difficulties encountered by peptide drug candidates for crossing the mammalian blood-brain barrier (BBB) (Egleton RD, Abbruscato TJ, Thomas SA, Davis TP Transport of opioid peptides into the central nervous system. J Pharm Sci 1998; 87(11):1433-9), as well as absorption after oral administration. (Borchardt R, Optimizing oral absorption of peptides using prodrug strategies. J Control

Release 1999;62(1-2):231-8) Because of this, ESP7 envisioned intrathecal administration and administration through other means could yield short duration or no analgesia. Additionally, the peptide endomorphin-2 does not have the full analgesic effect of morphine.

Morphine is a relatively complex organic molecule, termed an alkaloid due to its positively charged nitrogen group, unlike the endogenous peptide endomorphin-2 which provided the analgesic moiety in ESP7. Morphine is a highly efficacious MOR-selective opioid analgesic and will cross the human BBB, as will its active metabolite morphine 6-glucuronide. (Stain-Texier F, Boschi G, Sandouk P, Scherrmann JM, Elevated concentration of morphine 6-beta-D-glucuronide in brain extracellular fluid despite low blood-brain barrier permeability. Br J Pharmacol 1999; 128(4):917-24)

Substance P, however, is a peptide. Chimeric hybrid molecules possessing an alkaloid moiety and a peptide moiety are unknown to the literature of analgesia and to clinical practice. Chimeric hybrid molecules possessing an alkaloid moiety to activate the human MOR and a peptide moiety to concurrently activate the human SP receptor (SPR) are unknown to the literature of analgesia and to clinical practice. Chimeric hybrid molecules comprised of one moiety with a chemically modified morphine molecule to activate

the human MOR and another moiety with a SP fragment to activate the human SPR are unknown to the literature of analgesia and to clinical practice. The method of inhibiting the development of opioid tolerance using such chimeric hybrid molecules is unknown to the literature of analgesia and to clinical practice.

Another major challenge is to design a molecule that will cross the BBB and produce analgesia in a living subject, while inhibiting tolerance development and dependence formation. Such a molecule should be structured in such a way as to activate simultaneously the MOR and SPR domains in the PNS and/or CNS. With respect to both morphine and SP, a variety of alkaloid morphine and SP peptide fragments can be synthesized, having potentially different pharmacological effects if bound to another moiety. No obvious method is known for the SP moiety to be cross-linked to a morphine alkaloid moiety in a fashion that the resulting molecule will allow simultaneous activation of both the MOR and SPR receptors. Chimeric hybrid molecules with a moiety comprised of a chemically modified morphine molecule to provide the method to transport active SP fragments across the mammalian BBB are unknown to the literature of analgesia and to clinical practice.

The novelty of the present invention is not predictable according to the teachings of Rothman [1, Rothman, R.B. (1992) A review of the role of anti-opioid peptides in morphine tolerance and dependence. Synapse 12, 129-138.] who has formulated models and mechanisms of morphine and opioid tolerance and dependence that are exclusively mediated by functional changes in receptors and peptide transmitter systems within the CNS. Notably, Rothman teaches that adaptive mechanisms of morphine tolerance and dependence involve CNS neuropeptide systems that normally mediate homeostatic responses to attenuate adverse physiological effects of prolonged morphine exposure. The present invention is a general class of chimeric hybrid conjugate molecules capable of engendering efficacious opioid-dependent analgesia without opioid tolerance development that is functionally dependent on simultaneous activation of MOR and SPR receptors within the CNS following parenteral administration outside the CNS and, as such, its pharmacological effects are intrinsically a function of this class of molecules to permeate the mammalian BBB as an intact chemical entity. Accordingly, the analgesic and anti-opioid tolerance properties of this general class of chimeric hybrid conjugate molecules are functionally linked to the chemical and pharmacological integrity of each of the receptor activating domains to effectively permeate the BBB within a capped covalently bonded linear sequence. According to the teachings of Rothman, it

is not intuitively obvious and predictable that molecules of the general class of chimeric hybrid conjugate molecules possessing an opioid analgesic moiety or principle capable of engendering clinically-efficacious, opioid-dependent, acute and chronic pain relief equivalent to that produced by the prototype opioid alkaloid morphine will activate homeostatic anti-tolerance mechanisms within the CNS. As such, the requirement for an intact chimeric hybrid conjugate molecule to permeate the mammalian BBB as an intact chemical entity to enable each of its MOR and SPR receptor activation domains to effect clinically efficacious opioid analgesia without tolerance development distinguishes the present invention as novel and unknown to the literature of CNS analgesic and anti-abuse drugs.

The novelty of the present invention is not predictable according to the teachings of Foran and coworkers [2, Foran, S.E., Carr, D.B., Lipkowski, A.W., Maszczynska, I., Marchand, J.E., Misicka, A., Beinborn, M., Kopin, A.S., & Kream, R.M. (2000) A substance P-opioid chimeric peptide as a novel non-tolerance forming analgesic, Proc. Natl. Acad. Sci. USA 97, 7621-7626] in reference to those of Rothman. Foran and coworkers teach that repeated administration of a chimeric peptide containing MOR and SPR receptor activating domains into the rat CNS produces opioid-dependent analgesia without tolerance development that is

functionally linked to its SPR activating domain. Because the present invention is a general class of chimeric hybrid conjugate molecules capable of engendering efficacious opioid-dependent analgesia without opioid tolerance development that is functionally dependent on simultaneous activation of MOR and SPR receptors within the CNS following parenteral administration outside the CNS, its pharmacological effects are intrinsically a function of this class of molecules to permeate the mammalian BBB as an intact chemical entity. As such, the requirement for an intact chimeric hybrid conjugate molecule to permeate the mammalian BBB as an intact chemical entity to enable each of its MOR and SPR receptor activation domains to effect clinically efficacious opioid analgesia without tolerance development is not predictable by the teachings of Foran and coworkers in reference to those of Rothman and distinguishes the present invention as novel and unknown to the literature of CNS analgesic and anti-abuse drugs.

The novelty of the present invention is not predictable according to the teachings of Nyberg and coworkers [Zhou, Q., Karlsson, K., Liu, Z., Johansson, P., Le Greves, M., Kiuru, A. & Nyberg, F. (2001) SP endopeptidase-like activity is altered in various regions of the rat central nervous system during morphine tolerance and withdrawal. Neuropharmacology **41**, 246-253.] in

reference to the teachings of Foran and coworkers and Rothman. Nyberg and coworkers teach that CNS metabolism of SP and SPR activating domains via SP-specific endopeptidase activity is altered following morphine tolerance development and significant increases in SP-specific endopeptidase activity may be responsible for compensatory physiological responses in opioid tolerant animals. Because the present invention is a general class of chimeric hybrid conjugate molecules capable of engendering efficacious opioid-dependent analgesia without opioid tolerance development that is functionally dependent on simultaneous activation of MOR and SPR receptors within the CNS following parenteral administration outside the CNS, its pharmacological effects are intrinsically a function of this class of molecules to permeate the mammalian BBB as an intact chemical entity. As such, the requirement for an intact chimeric hybrid conjugate molecule to permeate the mammalian BBB as an intact chemical entity to enable each of its MOR and SPR receptor activation domains to effect clinically efficacious opioid analgesia without tolerance development without altering compensatory SP-metabolizing systems is not predictable by the teachings of Nyberg and coworkers in reference to those of Foran and coworkers and Rothman and distinguishes the present invention as novel and unknown to the literature of CNS analgesic and anti-abuse drugs.

The novelty of the present invention as a general class of chimeric hybrid conjugate molecules capable of engendering efficacious opioid-dependent analgesia without tolerance development that is functionally dependent on BBB transport is not predictable according to the teachings of Syvanen and coworkers [Syvanen, S., Xie, R., Sahin, S. & Hammarlund-Udenaes, M. (2006) Pharmacokinetic consequences of active drug efflux at the blood-brain barrier. Pharm. Res. 23, 705-717] who studied influx and efflux processes of morphine and morphine-glucuronides in relation to their BBB permeability properties and brain concentrations. Syvanen and coworkers teach that efficacious BBB permeation is determined by a combination of influx hindrance (a gatekeeper function in the luminal membrane that is functionally linked to P-glycoprotein activation) and efflux enhancement by transporters that pick up molecules on one side of the luminal or abluminal membrane and release them on the other side. The production of opioid-dependent analgesia for acute and chronic pain indications via a facilitative method of BBB transport of morphine and morphine congeners by covalently bonded heterologous SPR activating domains as found in the structure of chimeric hybrid conjugate molecules is not predictable by the general principle of BBB permeation by morphine and morphine congeners codified by Syvanen and coworkers. Conversely, the production of opioid-dependent analgesia for acute and chronic pain indications via a

facilitative method of BBB transport of SP or non-peptide SPR activating domains by covalently bonded heterologous morphine, morphine congeners, and opioid peptide MOR activating domains as found in the structure of chimeric hybrid conjugate molecules is not predictable by the teachings of Syvanen and coworkers.

Presently there also are no analgesic opioid molecules or chimeras that have been developed that achieve effective analgesia for mammalian acute or chronic pain without significant tolerance development and dependence formation.

Objects and Advantages. I have invented novel and useful methods employing heretofore unknown morphine-SP hybrid chimeras, as I have described below. Several objects and advantages of my present invention are:

a. a method for using a molecule that can be dosed to produce effective analgesia in a living subject, *i.e.*, a mammal (an animal class which includes humans), while inhibiting tolerance development;

b. a method for using a molecule that can be dosed to produce effective analgesia in a living subject while inhibiting dependence formation;

c. a method for using a molecule that can be dosed to produce effective opioid analgesia and that can be

administered through a variety of methods of clinical administration, including oral, systemic and epidural ~~intrathecal~~ administration;

d. a method for using a molecule that can be dosed to produce effective opioid analgesia without significant restriction on its effective dosage and time-effect relationship within the CNS due to metabolism in the blood stream;

e. a method for using a molecule that can be dosed to yield effective opioid analgesia with a reduction in the likelihood of undesirable side effects;

f. a method for using a molecule that can be dosed to produce effective opioid analgesia with a reduction in the likely severity of undesirable side effects that become manifested by the patient;

g. a method for using an opioid analgesic that can be dosed for administration to children without undue tolerance development;

h. a method for using an opioid analgesic that can be dosed for administration to children without undue dependence formation; and

i. a method for using an opioid analgesic suitable for PCA in the treatment of chronic and/or acute pain.

Additional objects and advantages of my present invention are:

a. to provide a method for treating pain with opioid analgesia and little or no opioid tolerance development;

b. to provide a method for treating pain with opioid analgesia and little or no opioid dependence formation;

c. to provide a method for treating pain with opioid analgesia with reduced likelihood of undesirable side effects;

d. to provide a method of opioid analgesia for PCA for acute and/or chronic pain; and

e. to provide a method of treating drug abuse by administering as a substitute for the abused drug an analgesic that elicits little or no tolerance development or dependency formation and thereafter adjusting the dosage as tolerance and/or dependence is modulated.

Still further objects and advantages will become apparent from a consideration of the following description of my invention.

Brief Summary of the Invention

~~The present invention provides a method of inhibiting opioid tolerance using novel chimeric hybrid molecules containing an opioid moiety of chemically modified morphine that binds to and activates an MOR and a SP peptide fragment moiety that binds to and activates an SPR.~~

The basis of the present invention is the construction of a general class of chimeric hybrid conjugate molecules capable of engendering efficacious opioid-dependent analgesia over a time course of administration without loss of analgesic potency. Within general definitions accepted by medical practitioners and research scientists, each and every representative example of this general class of chimeric hybrid conjugate molecules is deemed capable of delivering analgesia without the development of opioid tolerance and as such, possesses intrinsic anti-tolerance properties that are functionally determined by its chemical structure. Furthermore, the general class of chimeric hybrid conjugate molecules, designed to contain an opioid analgesic moiety or principle capable of engendering opioid-dependent analgesia without opioid tolerance development, possesses well established, clinically-efficacious, pharmacological properties for acute and chronic pain relief that are operationally defined by those of the prototype opioid alkaloid morphine. As such, a general class of chimeric hybrid conjugate molecules capable of

engendering efficacious, morphine-like, opioid-dependent analgesia for a variety of clinically defined pain indications without tolerance development is novel and unknown to the literature of CNS analgesic and anti-abuse drugs.

~~The present invention utilizes a family of chimeric hybrid molecules in which the alkaloid morphine or its active metabolite morphine-6 glucuronide are by design carriers of active SP peptide fragments across the mammalian BBB. I have designed this heretofore unknown family of hybrid, chimeric molecules with unique molecular hinges. This novel family of chimeric hybrid compounds can provide opioid analgesia in living subjects while inhibiting tolerance development and dependence formation. These chimeric hybrid compounds can also be used for drug abuse treatment. The hybrid alkaloid/peptide analgesics may be administered systemically, intrathecally or more preferably, orally.~~

~~In one embodiment, the independent functional domains consisting of chemically modified morphine and a SP fragment are covalently cross linked through the four carbon organic molecule succinic acid. In another embodiment, the independent functional domains consisting of chemically modified morphine and a SP fragment are covalently cross linked through the four carbon organic molecule~~

~~gamma-hydroxy-butyric acid. In another embodiment, the independent functional domains consisting of chemically modified morphine and a SP fragment are covalently cross linked through the six carbon carbohydrate d-glucuronic acid. The use of three such molecules, succinic acid, gamma-hydroxy-butyric acid, and d-glucuronic acid, as molecular hinges to cross link two active pharmacological domains of disparate chemical nature, i.e., a multi ringed opioid alkaloid structure and a linear peptide structure, is not intuitively obvious or predictable from the prior art. The use of succinic acid, gamma hydroxy butyric acid, and d glucuronic acid, as molecular hinges to cross link a pharmacologically active peptide to a pharmacologically active opioid is novel and unknown to the literature of analgesia and to clinical practice.~~

~~The chimeric hybrid molecule may be designed to have a plurality of SP moieties consisting of pharmacologically active COOH terminal fragments of SP and a plurality of opioid alkaloid moieties consisting of morphine chemically modified at its 6'hydroxyl group. The plurality of opioid moieties are each designed to bind to and activate an MOR. The plurality of SP fragments are each designed to bind to and activate an SPR. Because the MOR- and SPR activating domains are of chemically different compositions, i.e., a multi ringed alkaloid structure~~

~~and a linear peptide structure, respectively, it is not intuitively obvious that they may be combined in a functionally active molecule. This is achieved, however, by incorporating a novel molecular hinge region consisting of succinic acid, or gamma-hydroxy butyric acid, or d-glucuronic acid. The existence of functionally active chimeric hybrid molecules, of internally differing chemical nature, combining MOR and SPR activating domains linked by a novel molecular hinge are unknown to the literature of analgesia and to clinical practice.~~

The invention provides a method for inhibiting the development of opioid tolerance using pharmaceutical compositions including hybrid alkaloid chimeric molecules and a pharmaceutically acceptable carrier useful for the treatment of pain. It represents methods of treating pain using novel hybrid alkaloid/peptide chimeric molecules containing an opioid and SP moiety designed to achieve coincident activation of populations of MORs and SPRs as a novel pain treatment without tolerance and dependence. The hybrid alkaloid/peptide analgesics may be administered systemically or more preferably, orally. ~~Solubility, absorption, and penetration through the human BBB will be markedly enhanced due to the hydrophilic properties of morphine. The invention therefore provides novel methods for treating pain using chemically modified morphine to serve both as an opioid analgesic as well as a~~

~~pharmaceutically acceptable carrier for SP peptide absorption and stability after systemic administration as well as penetration through the human BBB.~~ In these novel attributes, the method of inhibiting opioid tolerance development using hybrid alkaloid chimeric molecules differs substantially from prior art including the use of peptide ESP7.

~~The method of inhibiting the development of opioid tolerance using novel hybrid alkaloid chimeric molecules encompassing three chemically disparate functional domains, i.e., a ringed alkaloid MOR activation domain, a peptide SPR activation domain, and a flexible organic acid hinge domain, is unknown to the preclinical and clinical literature of pain and analgesia.~~

A desired objective of the present invention is that the hybrid alkaloid/peptide chimeric molecules can be administered to produce clinically efficacious opioid analgesia with little or no development of opioid tolerance. With little or no tolerance development, escalating dosages will not be required to achieve the same pain killing effect and opioid dependence formation and undesirable side effects associated with escalating opioid dosages will be avoided or markedly reduced.

Detailed descriptions of one or more embodiments of the invention are described below. The novelty of the invention, as amply described above, will be apparent from the detailed description of structure and synthesis and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. All technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Unless expressly stated otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The examples of embodiments are for illustration purposes only. All patents and publications cited in this specification are incorporated herein by reference.

Brief Description of the Drawings

~~Fig. 1 illustrates two domains of the morphine nucleus, one being a conjugation domain useable to synthesize the chimeric hybrid compounds and the other being the active domain that activates the MOR.~~

Fig. 1 illustrates schematically how a chimeric hybrid conjugate molecule is constructed of three linked components that that combine any non-peptide opioid with any active fragment of SP.

~~Fig. 2 illustrates schematically how a chimeric hybrid molecule is constructed of three, linked components, i.e., a morphine nucleus, a linker-hinge and an SP fragment.~~

Fig. 2 illustrates schematically how a chimeric hybrid conjugate molecule is constructed of three linked components that combine any MOR-preferring opioid peptide with any non-peptide SPR activating domain.

Detailed Description of the Invention

Description - Figs 1 and 2. The present invention provides a method of inhibiting the development of opioid tolerance using hybrid alkaloid chimeric molecules having an MOR binding and activation moiety and an SPR binding and activation moiety. ~~The hybrid alkaloid chimeric molecules are designed to bind to and activate populations of MORs and SPRs located primarily within the human CNS, but also in the human PNS, involved in pain mediation and analgesic responses. While the alkaloid morphine and the peptide SP frequently exhibit slight cross reactivity to other opioid and tachykinin receptor types, respectively, they are generally characterized, as exhaustively detailed in the literature, by a very high degree of affinity for the MOR and SPR, respectively. The preservation of independent binding and~~

~~activation moieties in one hybrid alkaloid/peptide molecule containing a multi-ringed alkaloid structure and a linear peptide structure, is not described in the prior art and distinguishes the present invention as novel and not evolving from prior invention.~~

~~The existence of functionally active chimeric hybrid molecules, of internally differing chemical nature, combining MOR- and SPR-activating domains linked by a novel molecular hinge are unknown to the literature of analgesia and to clinical practice. Because the MOR- and SPR activating domains are of chemically different compositions, i.e., a multi-ringed alkaloid structure and a linear peptide structure, respectively, it is not intuitively obvious that they may be combined in a functionally active molecule.~~

~~I have achieved this by design and incorporation of a novel chemical linker hinge region consisting of succinic acid, or gamma hydroxy butyric acid, or d glucuronic acid, to connect within a single molecule an alkaloid MOR activation domain and a peptide SPR- activation domain that are modified to be compatible with that hinge. The design of novel hybrid alkaloid chimeric molecules encompassing three chemically disparate functional domains, i.e., a ringed alkaloid MOR activation domain, a peptide SPR activation domain, and a flexible organic acid hinge domain, is unknown to the preclinical and clinical literature of pain and~~

~~analgesia. The use of such hybrid alkaloid chimeric molecules to inhibit the development of opioid tolerance is unknown to the preclinical literature and clinical literature of pain and analgesia.~~

~~The chimeric multi ringed alkaloid structure of morphine linked to the linear peptide structure of SP is illustrated in Fig. 1 and Fig. 2. Fig. 1 illustrates that a morphine nucleus can be considered as divided into two domains, one of which is a conjugation domain 2 useable to synthesize the chimeric hybrid compounds from the 6'OH position on the morphine nucleus and the other of which is an active domain 1 that activates the MOR. Fig. 2 illustrates schematically how a chimeric hybrid molecule is constructed of three interlocking components, the alkaloid morphine nucleus 3, a chemical linker hinge 4, and a peptide SP fragment 5. The chemical linker hinge 4 links to the alkaloid morphine nucleus 3 at its 6'OH position. The chemical linker-hinge 4 also links to the peptide SP fragment 5. The linker-hinge allows the N terminal opioid receptor binding moiety or active domain of the morphine nucleus fragment of the hybrid chimeric molecule be able to activate an MOR and the C terminal SP receptor agonist binding moiety of the SP fragment to be able to activate an SPR.~~

~~The method employs chimeric hybrid molecules that may be designed to have a plurality of SP moieties consisting of pharmacologically active COOH terminal fragments of SP and a plurality of opioid alkaloid moieties consisting of morphine chemically modified at its 6'hydroxyl group. The plurality of opioid moieties are each designed to bind to and activate an MOR. The plurality of SP fragments are each designed to bind to and activate an SPR.~~

Fig. 1 depicts chimeric hybrid conjugate molecules that combine *any non-peptide opioid with any active fragment of SP, or any peptide, for* production of opioid-dependent analgesia for acute and chronic pain indications without tolerance development via *transport across the BBB* that are constructed as capped, electrically neutral, linear sequences with the non-peptide opioid covalently bonded to the N-terminal end of the SP fragment through a 4-6 carbon molecular linker, or according to the teachings of Schiller [Schiller, P.W. (2005) Opioid peptide-derived analgesics. A.A.P.S. J. 7, E560-567], a more complex heterocyclic structure, and containing a neutral amide group at the C-terminal end of the SP fragment. Fig. 1 depicts the construct of a linear chemical structure within the general class of chimeric hybrid conjugate molecules capable of simultaneous activation of MOR and SPR receptors within the CNS that contains a representative member of the morphinan, benzomorphan, or phenylpiperidine classes of non-

peptide opioid alkaloid in covalent linkage to a representative member of the class of 4-6 carbon or more complex heterocyclic molecular linker in covalent linkage to a representative member of the class of biologically active fragments of SP that include SP 3-11, SP 4-11, SP 5-11, SP 6-11, and SP 7-11, and their chemically modified congeners.

Representative candidate molecules chosen from the morphinan, benzomorphan, or phenylpiperidine classes of non-peptide opioid alkaloids, 4-6 carbon or more complex heterocyclic molecular linkers, and biologically active fragments of SP are listed in Table 1 and one of each may be covalently incorporated into the linear sequences of chimeric hybrid conjugate molecules according to guidelines gleaned from the teachings of Portoghese and coworkers [[Portogese, P.S. (2001) From models to molecules: opioid receptor dimers, bivalent ligands, and selective opioid receptor probes. J. Med. Chem. 44:2259-69; Bolognesi, M.L., Ojala, W.H., Gleason, W.B., Griffin, J.F., Farouz-Grant, F., Larson, D.L., Takemori, A.E. & Portoghese, P.S. (1996) Opioid antagonist activity of naltrexone-derived bivalent ligands: importance of a properly oriented molecular scaffold to guide "address" recognition at kappa opioid receptors. J. Med. Chem. 39, 1816-1822], Cascieri and Liang [Cascieri, M.A & Liang, T. (1983) Characterization of the substance P receptor in rat brain

cortex membranes and the inhibition of radioligand binding by guanine nucleotides. J Biol. Chem. 258, 5158-5164], and Mantyh and coworkers [Mantyh, P.W., Gates, T., Mantyh, C.R. & Maggio, J.E. (1989) Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. J. Neurosci. 9, 258-279.26] in reference to those of Liederer and coworkers [Liederer, B.M., Fuchs, J., Vander Velde, D., Siahaan, T.J. & Borchardt, R.T (2006) Effects of amino acid chirality and the chemical linker on the cell permeation characteristics of cyclic prodrugs of opioid peptides. J Med Chem. 49, 1261-1270] and Schiller.

Table 1: Representative molecules covalently incorporated into the linear sequences of chimeric hybrid conjugate molecules that combine any non-peptide opioid with any active fragment of SP to produce opioid-dependent analgesia for acute and chronic pain indications without tolerance development via transport across the BBB.

Peptide sequences are listed under the appropriate SEQ. ID No.

1. Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2
2. Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2
3. Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2
4. Gln-Phe-Phe-Gly-Leu-Met-NH2

5. Phe-Phe-Gly-Leu-Met-NH2

6. Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-dNorLeu-NH2

7. Pro-Gln-Gln-Phe--Gly-Leu-dNorLeu-NH2

8. Lys-Pro-Gln-Gln-Phe-dTryp-Gly-Leu-dNorLeu-NH2

9. Pro-Gln-Gln-Phe-dTryp-Gly-Leu-dNorLeu-NH2

Non-peptide opioid alkaloids	**Molecular linkers**	**Active fragments of substance P**	**SEQ. ID NO.**
Morphine*7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol*	Succinic acid ethane-1,2-dicarboxylic acid	substance P 3-11 KPQQFFGLM-NH2	1.
Dihydromorphine*7,8-dihydro-4,5-epoxy-17-methylmorphinan-3,6-diol*	Gamma-hydroxybutyric acid	substance P 4-11 PQQFFGLM-NH2	2.
Oxymorphone*4,5-α-epoxy-14-hydroxy-17-methyl-morphinan-6-one*	d-glucuronic acid	substance P 5-11 QQFFGLM-NH2	3.
Oxycodone *4,5-α-epoxy-14-hydroxy-3-methoxy-17-methyl-morphinan-6-one*	l-glucuronic acid	substance P 6-11 QFFGLM-NH2	4.
Hydrocodone4,5a-Epoxy-3-methoxy-17-methylmorphinan-6-one	oxaloacetic acid	substance P 7-11 FFGLM-NH2	5.
Pentazocine 2-hydroxy-5,9-dimethyl-2-(3-methylbuten-2-yl)-6,7-benzomorphanium	alpha ketoglutaric acid 2-Oxopentanedioic acid	D-nor-leu substance P 3-11 KPQQFFGLd-nor-L-NH2	6.
Cyclazocine 2-cyclopropylmethyl-5,9-	inositol cis-1,2,3,5-trans-4,6-cyclohexanehexol	D-nor-leu	7.
	tetrahydroisoquinoline-3-carboxylic acid		

dimethyl-2'-hydroxy-6,7-benzomorphan		substance P 4-11 PQQFFGLd-nor-L-NH2	
SufentanilN-[(4-(Methoxymethyl-1-(2-(2-thienyl)ethyl)-4-piperidinyl)]-N-phenylpropanamide		D-nor-leu, D-tryp substance P 3-11 KPQQFdWGLd-nor-L-NH2	8.
Carfentanil*4((1-oxopropyl)phenylamino)-1-(2-phenylethyl)-4-piperidinecarboxylic acid, methyl ester*		D-nor-leu, D-tryp substance P 4-11 PQQFdWGLd-nor-L-NH2	9.

Table 1

Fig. 2 depicts the construct of chimeric hybrid conjugate molecules that combine *any MOR-preferring opioid peptide, or for that matter any peptide, with any non-peptide SPR activating domain* for production of opioid-dependent analgesia for acute and chronic pain indications without tolerance development via transport across the BBB that are constructed as capped, electrically neutral, linear sequences with acetylation of the N-terminal of the opioid peptide that is covalently bonded at the C-terminal end to the non-peptide SPR activating domain through a 4-6 carbon molecular linker, or according to the teachings of Schiller a more complex heterocyclic structure. Figure 2 depicts the construct of a linear chemical structure within the general

class of chimeric hybrid conjugate molecules capable of simultaneous activation of MOR and SPR receptors within the CNS that contains a representative member of the class of MOR-preferring opioid peptide in covalent linkage to a representative member of the class of 4-6 carbon or more complex heterocyclic molecular linker in covalent linkage to a representative member of the class of non-peptide SPR activating domain.

Table 2: Representative molecules covalently incorporated into the linear sequences of chimeric hybrid conjugate molecules that combine any MOR-preferring opioid peptide with any non-peptide SPR activating domain for production of opioid-dependent analgesia for acute and chronic pain indications without tolerance development via transport across the BBB.

Peptide sequences are listed under the appropriate SEQ. ID No.

10. Ac-Tyr-Gly-Gly-Phe-Met
11. Ac-Tyr-Gly-Gly-Phe-Met-Arg-Phe
12. Ac-Tyr-dAla-Gly-Phe-Met
13. Ac-Tyr-Gly-Gly-Phe-Leu
14. Ac-Tyr-Gly-Gly-Phe-Leu-Arg-Gly-Leu
15. Ac-Tyr-dAla-Gly-Phe-Leu

16. Ac-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys

17. Ac-Tyr-Pro-Phe-Phe

Mu opioid receptor-preferring opioid peptides	**SEQ ID NO.**	**Molecular linkers**	**Non-peptide substance P receptor activating molecules**
N-acetyl methionine enkephalin Ac-YGGFM	10.	Succinic acid ethane-1,2-dicarboxylic acid	L-733,061 (partial agonist) (5S,6R)-6-alkyl-5-benzyloxy-2-piperidinone
N-acetyl methionine enkephalin-Arg-Phe Ac-YGGFMRF	11.	Gamma-hydroxybutyric acid	CP-99,994 (partial agonist) (+)-(2S,3S)-3-(2-methoxybenzyl amino)-2-phenylpiperidine
		d-glucuronic acid	
N-acetyl, D-ala2, methionine enkephalin Ac-YdAGL\FM	12.	l-glucuronic acid	
		oxaloacetic acid	
N-acetyl leucine enkephalin Ac-YGGFL	13.	alpha ketoglutaric acid 2-Oxopentanedioic acid	RP67580 (partial agonist) [imino 1 (methoxy-2-phenyl)-2 ethyl]-2 diphenyl 7,7 perhydroisoindolone-4 (3aR, 7aR)
N-acetyl leucine enkephalin-Arg-Gly-Leu Ac-YGGFLRGL	14.	inositol cis-1,2,3,5-trans-4,6-cyclohexanehexol	
N-acetyl,D-ala2, leucine enkephalin Ac-YdAGFL	15.	tetrahydroisoquinoline-3-carboxylic acid	
N-acetyl dynorphin A	16.		

(1-13) Ac-YGGFLRRIRPKLK			
N-acetyl endomorphin 2 Ac-TPFF	17.		

Table 2

~~I refer to the following amino acid sequences using the Seq. Id. Nos. below:~~

~~SEQ. ID. NO.~~	~~SEQUENCE~~
~~1~~	~~Lys Pro Gln Gln Phe Phe Gly Leu Met~~
~~2~~	~~Gln Gln Phe Phe Gly Leu Met~~
~~3~~	~~Phe Phe Gly Leu Met~~

~~Nine preferred embodiments of chimeric hybrid analgesics which the method of the present invention can employ are listed in table 1~~

~~Table 1:~~

~~Embodiment #~~	~~μ receptor agonist~~	~~Hinge~~	~~SP receptor agonist~~	~~Sequence~~
~~1~~	~~Morphine~~	~~D-Glucuronic Acid~~	~~N-AcetylsubstanceP[3-11]: Ac-KPQQFFGLM-NH2~~	~~SEQ. ID. NO. 1~~
~~2~~	~~Morphine~~	~~D-Glucuronic Acid~~	~~Substance P [5-11]: QQFFGLM-NH2~~	~~SEQ. ID. NO. 2~~
~~3~~	~~Morphine~~	~~D-Glucuronic Acid~~	~~Substance P[7-11]: FFGLM-NH2~~	~~SEQ. ID. NO. 3~~
~~4~~	~~Morphine~~	~~Succinic acid~~	~~N-AcetylsubstanceP[3-~~	~~SEQ. ID. NO. 1~~

			~~11]: Ac-KPQQFFGLM-NH2~~	
~~5~~	~~Morphine~~	~~Succinic acid~~	~~Substance P [5-11]: QQFFGLM-NH2~~	~~SEQ. ID. NO. 2~~
~~6~~	~~Morphine~~	~~Succinic acid~~	~~Substance P[7-11]: FFGLM-NH2~~	~~SEQ. ID. NO. 3~~
~~7~~	~~Morphine~~	~~Gamma-OH Butyric Acid~~	~~N-AcetylsubstanceP[3-11]: Ac-KPQQFFGLM-NH2~~	~~SEQ. ID. NO. 1~~
~~8~~	~~Morphine~~	~~Gamma-OH Butyric Acid~~	~~Substance P [5-11]: QQFFGLM-NH2~~	~~SEQ. ID. NO. 2~~
~~9~~	~~Morphine~~	~~Gamma-OH Butyric Acid~~	~~Substance P[7-11]: FFGLM-NH2~~	~~SEQ. ID. NO. 3~~

Advantages of The Present Invention.

The advantages of morphine as an analgesic that can cross the BBB are well known to the literature. The advantages of simultaneous activation of an MOR and SPR to modulate the activation of the MOR and to reduce or eliminate tolerance development and dependence formation are also known from the literature, such as a prior invention of mine (U.S. Patent 5,891,842) and the work of colleagues of mine and I identified above relating to ESP7.

From the description above, a number of advantages of my method of inhibiting opioid tolerance development using chimeric hybrid analgesic molecules becomes evident:

a. the method will inhibit tolerance development while being dosed to provide morphine opioid analgesia;

b. the method will inhibit dependence formation while being dosed to provide morphine opioid analgesia;

c. the method can be used by means of administration of the molecules through a variety of methods of clinical administration, in addition to intrathecal administration;

d. the method will not have the significant dosage and time-effect restrictions of peptides due to metabolism in the blood stream;

e. because of the modulation of an MOR by SPR activation, an escalating dosage typical of morphine is not required;

f. because the escalating dosage typical of morphine is not required, the likelihood and severity of undesirable effects associated with escalating morphine dosage will be reduced; and

f. the method can be used to administer a chimeric hybrid analgesic molecule as a substitute for an abused opioid drug and, because the molecule elicits little or no tolerance development or dependency formation, its dosage can thereafter be adjusted as tolerance and/or dependence is modulated.

Further advantages will become apparent to those skilled in the art.

Making My Invention. The present invention can be made by a person skilled in the art, as follows.

In light of the work of Syvanen and coworkers cited above, the teachings of Liederer and coworkers provide us with guidelines by which to construct a general class of chimeric hybrid conjugate molecules capable of opioid-dependent analgesia for acute and chronic pain indications that combine any non-peptide opioid with any active fragment of SP, or any peptide, for transport across the BBB. Liederer and coworkers teach that low BBB permeation is functionally linked to strong substrate activity for P-glycoprotein and efflux transporters in this biological barrier that is markedly enhanced for a variety of tested opioid peptide analogs sharing a common covalent cyclical structure. In contrast, capped, electrically neutral, linear derivatives of a variety of opioid peptide analogs with acetylation of the N-terminal and amidation of the C-terminal ends display efficacious permeation of the BBB via low substrate activity for P-glycoprotein and efflux transporters in this biological barrier.

Application of guidelines derived from the teachings of Liederer and coworkers in reference to the teachings of Syvanen and coworkers will enable any person skilled in the art to which it

pertains to make and use the invention commensurate in scope with Claim 1, i.e., a general class of chimeric hybrid conjugate molecules capable of producing opioid-dependent analgesia for acute and chronic pain indications without tolerance development via simultaneous activation of MOR and SPR receptors within the CNS. Chimeric hybrid conjugate molecules that combine any non-peptide opioid with any active fragment of SP, or any peptide, for production of opioid-dependent analgesia for acute and chronic pain indications without tolerance development via transport across the BBB are constructed as capped, electrically neutral, linear sequences with the non-peptide opioid covalently bonded to the N-terminal end of the SP fragment through a 4-6 carbon molecular linker and containing a neutral amide group at the C-terminal end of the SP fragment. Chimeric hybrid conjugate molecules that combine any opioid peptide, or for that matter any peptide, with any non-peptide SP receptor activating domain for production of opioid-dependent analgesia for acute and chronic pain indications without tolerance development via transport across the BBB are constructed as capped, electrically neutral, linear sequences with acetylation of the N-terminal of the opioid peptide that is covalently bonded at the C-terminal end to the non-peptide SPR activating domain through a 4-6 carbon molecular linker. Finally, the teachings of Schiller in reference to those of Syvanen and coworkers and Liederer and coworkers demonstrate a

permissive chemical heterocyclic substitution in the internal domains of capped linear opioid peptide sequences that allow for efficacious BBB permeation, thereby providing validation for our specification indicating d-glucuronic acid, as a representative example of a closed-ring carbon structure, as an appropriate 6 carbon linker connecting linear MOR and SPR receptor activating domains within chimeric hybrid conjugate molecules.

The production of opioid-dependent analgesia for acute and chronic pain indications via a facilitative method of BBB transport of morphine and morphine congeners by covalently bonded heterologous SPR receptor activating domains or conversely, of BBB transport of SP fragments or non-peptide SPR activating domains by covalently bonded heterologous morphine, morphine congeners, and opioid peptide MOR activating domains, requires maintenance of opioid and SP activities in chemically-modified structures of chimeric hybrid conjugate molecules. The teachings of Portoghese and coworkers in reference to those of Liederer and coworkers and Schiller provide specific indications for maintaining opioid activity following chemical modification of the multi-ringed non-peptide structures characteristic of morphinans, benzomorphans, and phenylpiperidines, as described for opioid peptide analogs. The construction of hybrid chimeric conjugates containing non-peptide opioids or chemically modified opioid peptide sequences are

consistent with guidelines provided by Portoghese and coworkers, established authorities in the synthesis and structure-function relationships of non-peptide opioids, in reference to the teachings of Liederer and coworkers and Schiller and will enable any person skilled in the art to which it pertains to make and use the invention commensurate in scope with Claim 1, i.e., a general class of chimeric hybrid conjugate molecules capable of simultaneous activation of MOR and SPR receptors within the CNS to produce opioid-dependent analgesia for acute and chronic pain indications without tolerance development.

In brief, the teachings of Portoghese and coworkers provide the following guidelines for preserving high affinity MOR activity for all non-peptide opioid domains found in the general class of chimeric hybrid conjugate molecules capable of simultaneous activation of MOR and SPR receptors within the CNS. Their teachings indicate that the A ring OH group at position 3 must be conserved during synthesis and/or conjugation to active SP fragments though a linker molecule. Consistent with the major body of published opioid research, conservation of the A ring OH group at position 3 is required for high affinity MOR activation. Thus, the A ring OH group at position 3 may be protected during synthesis or conjugation via covalent linkage to well recognized blocking groups that include Acetyl or T-butyl moieties.

Following synthesis or construction of chimeric hybrid conjugates the Acetyl or T-butyl moieties are removed by gentle chemical treatment yielding non-peptide chemical moieties with a free A ring OH group at position 3.

The teachings of Portoghese and coworkers also indicate that the B ring OH group at position 6 of morphine or an equivalent position on the morphinan or benzomorphan multi-ringed structure is an appropriate site for chemical modification due to its location at a point distal to the obligate A ring OH group at position 3 of morphine or an equivalent position on the morphinan or benzomorphan multi-ringed structure. Chemical modification and linkage of the non-peptide opioid domain of molecules of the general class of chimeric hybrid conjugate molecules capable of simultaneous activation of MOR and SPR receptors within the CNS at a position spatially separated and distal to the obligate A ring OH group will permit binding in a sterically unhindered fashion to the MOR. The B ring OH group at position 6 of morphine or an equivalent position on the morphinan or benzomorphan multi-ringed structure may be further oxidized to a keto group with full retention of opioid activity. OH and keto groups are generally employed as chemical moieties capable of covalently linking discrete chemical entities through ester or ether chemistry. Finally, the teachings of Portoghese and coworkers indicate that

multiple positions of the B ring, including the OH group at position 6 of morphine, or an equivalent position on the morphinan or benzomorphan multi-ringed structure, may be chemically modified without effecting opioid activity mediated by the obligate A ring OH group.

The construction of hybrid chimeric conjugates containing non-peptide opioids or chemically modified opioid peptide sequences are consistent with guidelines provided by Portoghese and coworkers, established authorities in the synthesis and structure-function relationships of non-peptide opioids, in reference to the teachings of Liederer and coworkers and Schiller and will enable any person skilled in the art to which it pertains to make and use the invention commensurate in scope with Claim 1, i.e., a general class of chimeric hybrid conjugate molecules capable of simultaneous activation of MOR and SPR receptors within the CNS to produce opioid-dependent analgesia for acute and chronic pain indications without tolerance development.

The teachings of Cascieri and Liang and Mantyh and coworkers provide specific indications for maintaining SP activity for C-terminal fragments of SP within a general class of chimeric hybrid conjugate molecules capable of producing opioid-dependent analgesia for acute and chronic pain indications without tolerance

development via simultaneous activation of MOR and SPR receptors within the CNS. The rules provided by Cascieri and Liang and Mantyh and coworkers are considered to be general rules for evaluating bioactivities of fragments of SP by established investigators in SP research. According to their teachings and consistent with generally accepted formulations, all fragments of SP maintaining a fully intact C-terminal peptide domain equal to or greater than 5 amino acids have been determined to possess biological activity using a variety of testing paradigms. In the present invention, biologically active fragments of SP include SP 3-11, SP 4-11, SP 5-11, SP 6-11, and SP 7-11. All biologically active SP fragments contain only one free alpha amino group that is located at a site distal to SPR recognition domain and is utilized as the point of linkage of all active fragments of SP within the structure of the class of chimeric hybrid molecules described in the present invention. In sum, the teachings of Cascieri and Liang and Mantyh and coworkers in reference to the teachings of Portoghese and coworkers, Liederer and coworkers, and Schiller provide guidelines that will enable any person skilled in the art to which it pertains to make and use the invention commensurate in scope with Claim 1, i.e., a general class of chimeric hybrid conjugate molecules capable of simultaneous activation of MOR and SPR receptors within the CNS to produce

opioid-dependent analgesia for acute and chronic pain indications without tolerance development .

The method uses chimeric hybrid analgesic molecules to inhibit the development of opioid tolerance. The separate MOR- and SPR-activating moieties are synthesized and purified or isolated from natural sources and then chemically cross-linked to form hybrid alkaloid/peptides chimeric molecules. All syntheses utilize well-established standard organic chemistry techniques and reagents. ~~SP peptide fragment moieties are synthesized prior to covalent attachment to the morphine nucleus (Fig. 1). For these purposes, a variety of peptide synthesis methods are common in the art, including synthesis using an automated peptide synthesizer and employing Fmoc amino acids. (Merrifield, Science 232: 241-247 (1986); Barany, et al, Intl. J Peptide Protein Res. 30: 705-739 (1987); Kent, Ann. Rev. Biochem. 57:957-989 (1988), and Kaiser, et al, Science 243: 187-198 (1989)) SP peptide fragments are purified to over 99% chemical purity using standard peptide purification techniques such as reverse phase high-pressure liquid chromatography (HPLC). The chemical structures of SP peptide fragments, purified by HPLC, are confirmed by mass spectroscopic analysis.~~

~~Morphine is chemically modified by covalent attachment at its 6'OH group to the hinge forming organic molecules described above: d-glucuronic acid, succinic acid, gamma hydroxy butyric acid. Chemically modified morphine derivatives, i.e., morphine-6-glucuronide, morphine-6-hemi-succinate, morphine-6 gamma-hydroxy butyrate, are covalently attached to SP peptide fragments using standard condensing agents such as water soluble carbodiimide (CDI).~~

~~Alternatively, SP peptide fragments are chemically modified by covalent attachment at their free amino groups to the hinge-forming organic molecules described above: d-glucuronic acid, succinic acid, gamma-hydroxy-butyric acid. Chemically modified SP peptide fragments, i.e., SP fragment-glucuronide, SP-fragment-hemi-succinate, SP fragment-gamma hydroxy butyrate, are covalently attached to morphine using standard condensing agents such as water soluble CDI.~~

Prior to pharmacological testing, the novel chimeric hybrid ~~alkaloid/peptide~~ molecules ~~comprising a cyclic alkaloid MOR-activating moiety and an SPR activating peptide moiety~~ (such as those in Tables 1 and 2) are purified to over 99% purity by standard chromatographic techniques such as reverse-phase HPLC. This represents less than about 1% chemical precursors or non-

peptide chemicals in the final preparations. The chemical structures of chimeric hybrid alkaloid/peptide molecules are confirmed by mass spectroscopic analysis. The chimeric hybrid molecules are then subjected to standard pharmacological testing.

Preclinically, a well-established method is used to assess the analgesic properties of the novel chimeric hybrid compounds, that being the tail flick test, which is administered to rats following parenteral or CNS administration. Additional tests of analgesic responsiveness include the paw withdrawal and hotplate tests, i.e., methods well-established as common in the art. Preclinical testing of analgesia and tolerance development is conducted by administration of the chimeric hybrid compounds over time and alternatively using opioid and SP blockers in well-established analgesic testing methods. Further preclinical and clinical testing is conducted in conformity with governmental drug regulations.

Having made the chimeric hybrid molecules, they are administered to inhibit the development of opioid tolerance through means of clinical administration of analgesia well known to persons skilled in the art.

<u>Using My Invention</u>. The present invention further provides methods of treating a mammal for relief of pain by administering a pharmaceutical composition (as described above) in order to produce analgesia in the subject/patient. The invention is used by persons skilled in the art, as follows: Pharmaceutical compositions of the invention are formulated to be compatible with their intended routes of administration, e.g., parenteral, intrademal, subcutaneous, injectable, intravenous, oral, intradermal, subcutaneous, transdermal (topical), transmucosal, <u>epidural</u> and rectal administration.

Solutions or suspensions suitable for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, sterile or bacteriostatic water, or phosphate buffered saline (PBS). In all cases, the compositions must be sterile and should be fluid to the extent that they are easily injectable by syringe. Proper fluidity may be maintained by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Preservation of chemical and pharmaceutical integrity is achieved by various antibacterial and antifungal agents: e.g., parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, etc. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., chimeric hybrid molecules) in the required dosage in an appropriate solvent with one or a combination of

ingredients enumerated above, as required, followed by filtered sterilization.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with binders and used in the form of tablets, troches, or capsules. Pharmaceutical binding agents, and/or adjuvant material can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide.

Suitable intradermal, subcutaneous, transdermal (topical), and transmucosal formulations include: gels, creams, solutions, emulsions, suspensions, carbohydrate polymers, biodegradable matrices thereof, vapors, mists, aerosols and other inhalants, and skin patches. Rectal formulations also include suppositories and enemas.

Examples of suitable pharmaceutical carriers for the various forms of administration include any of the standard pharmaceutically accepted carriers known to those of ordinary skill in the art. Examples of pharmaceutical carriers include but are not limited to buffered saline solution, water, emulsions, various wetting agents, tablets, coated tablets and capsules. Besides an effective amount of the compounds described in the present invention, pharmaceutical compositions may include suitable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers. Examples of optional ingredients which may be included in the pharmaceutical compositions of the present invention include antioxidants; low molecular weight polypeptides; proteins such as serum albumin, gelatin or immunoglobulins; amino acids such as glycine; chelating agents; sugar alcohols.

Because of the modulation of opioid tolerance and dependence, the invention may also be used for drug abuse intervention through administration of one or more embodiments of the chimeric hybrid analgesics which are the subjects of the invention in substitution for the drug to which the patient became tolerant and/or on which the patient became dependent.

Conclusions, Ramifications and Scope. The reader thus will see that my invention provides a novel and useful method for

inhibiting the development of opioid tolerance using novel chimeric hybrid molecules containing an opioid moiety of chemically modified morphine that binds to and activates the MOR and a SP peptide fragment moiety that binds to and activates the SPR, to produce opioid analgesia in a living subject with little or no tolerance development and dependence formation.

While my description contains many specifications, these should not be construed as limitations on the scope of my invention, but rather as an exemplification of one or more of the preferred embodiments of my invention. Other variations are possible. Accordingly, the scope of my invention should be determined by the appended claims and their legal equivalents and not by the embodiments illustrated in the foregoing description.